

# Recruitment of Phagocytizing Cells into the Respiratory Tract as a Response to the Cytotoxic Action of Deposited Particles

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Recruitment of phagocytizing cells into the lower respiratory tract plays a very important role in the pulmonary dust clearance, depending both on the number of particles deposited therein and on their aggressiveness. The higher cytotoxicity of the particles, the greater the number of such cells recruited and the higher the contribution of the neutrophilic leukocytes (NL) into the free cellular population of airways which normally is represented chiefly by alveolar macrophages (AM). Adaptation of the alveolar dust phagocytosis to properties of inhaled particles operates through autoregulation of this process in which a key role is played by macrophage breakdown products (PMB). A series of experiments *in vitro* and *in vivo* showed that PMB stimulate AM and NL, enhance their recruitment into airways with a dose-dependent increase of the NL/AM ratio, promote recruitment of their precursors via blood and replenishment of such precursor reserves. The most active factor of the PMB appears to be lipidic by nature. The variability between individuals and between groups of alveolar phagocytosis response to particles of a given cytotoxicity may be due to differences of the host's neurohormonal status. It was shown that influencing the latter significantly shifts response to a standard dose of the PMB.

The free surface of the respiratory tract, especially that of lung acinus, always contains a certain number of cells not connected with the epithelial lining and is represented in normal conditions mainly by pulmonary macrophages. These "free macrophages" are more or less easily washed from the airways by bronchoalveolar lavage. Repeated lavages with the use of some additional techniques favoring defixation of free macrophages made it possible to give the best quantification of this cell population. In healthy rats not exposed to harmful influences, this population is about  $1.1 \times 10^7$  cells/g lung weight (1,2). According to the same authors, this population is increased approximately 2-fold when particles of carbon, barium sulfate or iron oxide are deposited in lungs, whereas the deposition of chrysotile asbestos particles increases the population 5-fold. It is this dependence of alveolar macrophages (AM) recruitment on the quality of the dust that will be the main subject of discussion. However, the same studies and earlier experiments (3, 4) show also dependence of the number of recruited AM on the number of particles deposited.

While enhanced AM recruitment, as a response to deposition of inhaled particles in lungs, is not in doubt, the role of this response as a mechanism of pulmonary clearance is not universally acknowledged (5-7). However,

there exist many arguments which, though indirect, on the strength of all evidence leave little doubt that alveolar phagocytosis does play a most important role in the elimination of dust particles from the alveolar region, especially that of particles of high cytotoxicity (e.g., silica particles).

## Recruitment of Phagocytizing Cells and Pulmonary Clearance

The involvement of recruited phagocytic cells in pulmonary clearance is suggested by the fact that the number of free AM, after reaching its maximum during the first 24 hr after particle deposition, then decreases with the elimination of dust from the lungs. The half-times of both processes practically coincide (3). Further, it was shown that the more cytotoxic the dust is, i.e., the more the macrophage is damaged by the particles engulfed by it, the greater is the penetration of these particles into the pulmonary interstitial tissue and lymph and the less efficient is their elimination from the lungs (8-13). On the other hand, the defense of the macrophage from damage by quartz dust, as, for example, by injection of polyvinylpyridene-*N*-oxide (PVPO) promotes lung clearance (14-16). A similar effect was observed when the resistance of the macrophage to different damaging exposures, including the cytotoxicity of quartz, had been increased on the

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background of adaptation of the organism to moderate physical training or to low concentrations of sulfurous anhydride (17,18) or to a weak antigenic challenge.

On the other hand, an increase of sensitivity of alveolar macrophages to the cytotoxicity of quartz caused by an enhanced accumulation of lipids in AM due to long-term peroral administration of fat promotes the retention of dust in the lungs (19). It is interesting to note that PVPO does not appear to influence the elimination of those particles which do not cause pronounced damage to the macrophage (20). All these data support the hypothesis (21,22) that the role of phagocytosis in pulmonary dust clearance increases the probability of the transportation of dust particles together with the phagocytizing cell along the surface of the acinus to the zone of action of the mucociliary escalator, as this cell is evidently incapable of migrating back into the lung interstitium. Hence, it is clear that the breakdown of the cell, leading to liberation of dust particles, again increases the probability of their penetration and retention.

When particles—primarily particles of high cytotoxicity—are deposited in lungs, the free cell population of airways is increased not only on account of alveolar macrophages but also of one more phagocytizing cell—the neutrophilic leukocyte (23–28).

This response is sometimes described as “inflammatory” (29), i.e., as a pathological rather than a normal one. It is, however, hardly expedient to draw a sharp line between a defense physiological mechanism of AM recruitment and the process of recruitment of neutrophil leukocytes (NL). The latter, as shown long ago in our laboratory, is a constant feature of a phagocyte response to quartz dust, even with minimal dust loads (18). The defense role of NL recruitment, i.e., the role promoting dust clearance, is not in doubt, in spite of the fact that the average number of dust particles observed by optical microscopy in a single NL seldom exceeds two, even after inhalation of dust in a high concentration, while under the same conditions many AM contain a practically uncountable number of dust particles. However, at lower concentrations of inhaled dust, the average particle load on a single AM is commensurable with that for a single NL, and our experiments show that in such conditions a sharply increased NL number can account for up to half of all particles phagocytized by the free cell population of the lungs (30,31).

## Cytotoxicity of Dust and the Characteristics of Alveolar Phagocytosis

For the past 14–15 years, our laboratory has been studying the characteristics of the alveolar phagocytosis response to short-term and long-term exposures to various dusts, mainly quartz. This response is characterized by the following permanently reproducible features:

(1) AM recruitment in response to quartz deposition

is much more pronounced than in response to deposition of dusts of low cytotoxicity.

(2) NL recruitment increases still more after both inhalation and intratracheal injection of quartz particles, so that the NL/AM ratio in lavage effluents is considerably higher than in the case of analogous exposure to particles of low cytotoxicity.

(3) The more the AM is susceptible to the damaging action of a standard quartz dust, the more both these features are. Factors mentioned in the above section that increased or decreased the resistance of AM influenced the total number of recruited phagocytes and the NL/AM ratio to the same degree to which they increased or decreased the percent of degenerated AM (18,19). As an example one may cite the mean group values of the latter index for rats exposed to quartz dust for 6 months on the background of subcutaneous PVPO injections or without such injections (16.6% and 54.8%,  $p < 0.001$ ) and the corresponding mean NL/AM values (0.59 and 2.40,  $p < 0.0001$ ), the total number of cells in lung wash-outs being  $3.43 \times 10^6$  and  $1.07 \times 10^7$  ( $p < 0.05$ ).

Note that this change and similar changes of phagocyte response due to inhalation of sulfurous anhydride lead to a decrease in retention of dust in lungs. Thus, the organism is able to limit AM recruitment, especially that of NL when an effective clearance can be assured at lower costs in cell resources. Such regulation of the response is even more expedient because recruitment of an unusually large number of cells to the free alveolar surface can, by creating hindrances to their draining through a considerably smaller surface of the acinus mouth (32), become by itself, not an elimination mechanism, but a cause of dust retention. For instance, when AM and NL recruitment was enhanced by the action of some biogenic stimulators (33) or of cold acclimatization (17), pulmonary dust retention increased.

On the other hand, a milder stimulation of this recruitment (33) or stimulation by the action of a dust of low cytotoxicity, which by itself causes a moderate AM and NL recruitment (34), reduces dust retention.

The correspondence between the above-mentioned peculiarities of alveolar phagocytosis response and the degree of macrophage damage was evident even in the absence of any organized factors influencing AM resistance. The same refers to inter-individual differences within a group of rats exposed (or not) to quartz inhalation. Thus, for the former we found that the NL/AM ratio ( $y$ ) dependence on the percentage of degenerated AM ( $x$ ) can be described by the equation,  $y = 0.2 + 0.35x - 0.001x^2 + 0.00001x^3$ .

All these facts definitely point to the existence of physiological control of phagocytizing cell recruitment into the lungs in response to dust particle deposition depending on their cytotoxicity, i.e., on the number of macrophages destroyed through the action of some products of macrophage breakdown. Such a hypothesis was put forward by one of us (17,18) and later received additional support in a series of experiments with

products of induced macrophage breakdown (30,31, 35-37).

Although we stress here the dependence of alveolar phagocytosis response on cytotoxicity, i.e., on the quality of dust, it is easy to note that the same hypothesis is quite applicable to the explanation of the dependence of this reaction on its quantity. The more particles deposited in lungs, the more AM can be destroyed by them (other conditions being equal), i.e., the more physiologically active macrophage breakdown products are formed. Let us note also that with the decrease of particle size, i.e., with the increase in their number (in a constant mass), the probability of engulfment of a given mass of inhaled material by a greater number of AM naturally increases. Besides, when the particle size decreases, the specific surface of engulfed dust is increased. The cytotoxic action of particles, especially quartz particles, is related to processes taking place on their surface. Thus, the probability of breakdown of a large number of AM increases and more products causing cell recruitment are formed (or liberated). Therefore, it is not surprising that the number of free AM depends to a larger degree on the number of deposited particles than on their total mass (1,38).

## Macrophage Breakdown and Control of Phagocytizing Cell Recruitment into Airways

The hypothesis of a possible connection between AM recruitment into the lungs and the action of some products of macrophage origin was also put forward by other researchers starting from different, most often speculative premises (39,40). A supposition of a similar influence of enzymes, liberated due to the AM breakdown by quartz dust, on NL recruitment was later expressed by Miller and Kagan (41). There are also some experimental data on production or liberation by alveolar macrophages of a chemotactic factor stimulating NL migration *in vitro* and *in vivo* (42,43).

Our experiments were performed on Wistar rats and CBA, C57BL, and BALB/c mice. The products of syngenetic macrophage breakdown (PMB) were injected intratracheally or intraperitoneally. The PMB used for this were obtained aseptically by triple freezing and thawing of the peritoneal exudate cells obtained 45 hr after intraperitoneal injection of a sterile mineral oil or normal saline; 82 to 86% of the cells consisted of macrophages.

The cytotoxic influence of mineral particles causes an influx of granulocytes not only into the respiratory tract as demonstrated in an experiment with triple IP injection of 30 mg of quartz or  $\text{TiO}_2$ . The exudate obtained 24 hr after the third injection of the titanium dioxide suspension contained only  $15.5 \pm 1.9\%$  of granulocytes (relative to all cells). That did not differ from the exudate composition after triple injection of the normal saline. At the same time, after a similar

injection of quartz suspension, this figure rose to  $32.0 \pm 2.8\%$  ( $p < 0.001$ ). Clearly degenerated cells constituted, correspondingly,  $7.0 \pm 1.7\%$  and  $50.0 \pm 2.4\%$  of all the exudate macrophages ( $p < 0.001$ ). Such attraction of granulocytes to the site of macrophage breakdown was modelled in intraperitoneal injection of artificial PMB in the dose corresponding to  $1.5 \times 10^8$  broken down macrophages/100 g body weight. In this case, the granulocytes rose to  $52.3 \pm 3.3\%$  ( $p < 0.001$ ). However, neither the rats exposed to the titanium dioxide dust by inhalation nor those who were not exposed to it showed any change in the total number of cells in the lung washing or in the NL/AM ratio under the influence of intraperitoneally injected titanium dioxide or quartz suspension or the artificial PMB.

Such changes of a marked local character were constantly observed when PMB had been intratracheally injected. Many such experiments were performed with different PMB doses. Control rats in this case were injected intratracheally with 1 mL of normal saline. The analysis of lung washings was conducted after 24 hr. In two experiments the animals were exposed also to  $\text{TiO}_2$  dust, which they inhaled in a chamber at  $50 \text{ mg/m}^3$  or  $85 \text{ mg/m}^3$  concentration 5 to 6 hr/day for 4 consecutive days, and the PMB was injected in  $3 \times 10^8$  doses immediately after the fourth inhalation, or in half doses, after the second and fourth inhalations. Here and further on we give the PMB doses per rat (of about 200 g body weight) in units corresponding to the number of cells destroyed; the PMB concentrations are given in the same units per milliliter of medium. All these experiments without exception showed increased recruitment of phagocytizing cells into the respiratory tract under the influence of PMB, with sufficiently large doses causing attraction of both AM and NL, while smaller ones caused only NL attraction. However, even when the doses were larger, the number of NL showed a greater increase in comparison with the control than the number of AM, which resulted in a higher NL/AM ratio at all doses than in the control groups. This ratio increased with an increase in dose.

Unlike the attraction of NL from blood into the respiratory tract, which increases almost linearly with the increase of attractant amount, AM recruitment is governed by the action of PMB rather than by a trigger mechanism or mechanisms causing two sharp increases of the AM count. One cannot eliminate the possibility of the existence of different sources and/or mechanisms of additional mobilization of AM into the alveoli, each of which has its own PMB dose range or its particular threshold dose. For example, we can suppose that the background number of free AM is provided mainly by migration of mature macrophages from the interstitial pool (38,44,45). It is hard to say to what degree this background migration is related to the attraction of cells by breakdown products of the macrophages with evident degeneration which are always present in the respiratory tract. The above-mentioned dependence of the number of AM in control rats on the percentage of

such macrophages indirectly proves that they also exhibit such an attraction, depending on the PMB dose. In this case an additional PMB injection in small doses may simply fail to give a noticeable effect on the background of individual fluctuations of the amount of endogenous PMB. However, the fact that high enough doses not only make this effect noticeable, but also act in a certain range on the "all-or-nothing" principle, allow us to think of "ejection" from the same pool of some subpopulation of precursor cells which have not yet reached the mature stage. The next threshold dose either acts in a similar way on a still less mature subpopulation or else causes a direct attraction of mononuclear phagocytes from the blood into the alveoli.

The possibility of such direct recruitment of monocytes under the influence of a sufficiently strong challenge is indicated by a number of data which though circumstantial and subject to different interpretations are, however, rather convincing in their totality (46-50). The supposition about the possible existence of different sources of AM recruitment in the state of relative rest and as a response to a pronounced challenge on the part of deposited particles was expressed elsewhere (2).

It must be stressed that both the quantity of and the ratio between different phagocytes in the lung washings are more reminiscent of the picture typical for the quartz dust reaction, the greater the PMB dose.

Table 1 shows the data from one of the experiments in which PMB was injected both in unexposed rats and those who previously inhaled  $\text{TiO}_2$  dust. This dust, which is of low cytotoxicity and even sometimes called inert, caused, in combination with additional PMB, the alveolar phagocytosis reaction of the response to quartz dust type. It is also easy to see that even without addition of exogenous PMB it caused (in comparison with the control) a phagocytic reaction with a certain increase in the NL/AM ratio. The mean value of clearly degenerated AM in control rats was  $9.6 \pm 0.7\%$ , while in animals exposed to the  $\text{TiO}_2$  dust it was  $16.0 \pm 1.2\%$  ( $p < 0.001$ ).

So, whether we speak of the cytotoxic action of quartz, of a considerably less pronounced damaging action (probably having a different etiology) on a cell of other dust particles, of a breakdown of part of the AM caused by different "natural" reasons in the respiratory tract of control rats or of an intratracheal injection of breakdown products caused by a rough physical action on the macrophages, we observe in every case a

change—similar in principle—of the free cell population of the respiratory tract, to a degree evidently dependent on the number of disintegrated macrophages.

## Influence of PMB on Phagocytizing Blood Cells

Although there remains some vagueness in the question of tissue macrophage origin in general and pulmonary macrophage origin in particular there is little doubt today that all these cells in the final count have a bone marrow origin (2,29,51). We can refer also to conclusive results of observations of chromosome changes in macrophages of bronchoalveolar lavage in patients after a successful bone marrow transplantation from persons of the opposite sex (52). Along with this, convincing experimental data show that the local cell reserve—the interstitial precursor cell pool—may be an immediate source of AM recruitment to the alveoli (44,45,53-55). This reserve provides, on the one hand, a certain independence of enhanced AM mobilization as a response to a short-term challenge from the systemic increase of hemopoiesis, which, it seems, is also characteristic for macrophages of other organs (56-60), and, on the other hand, creates necessary conditions for maturing a functionally valid AM alongside with its gradually acquiring a number of morphological and biochemical features.

These considerations give prime importance to the question of the influence of PMB on the production of granulocytes and monocytes by the hemopoietic tissue and their mobilization via blood. The results of an experiment in which rats were injected IP three times in 3 days with a PMB dose corresponding to  $1.5 \times 10^8$  broken-down macrophages/100 g body weight are shown in Table 2. The changes in cell composition of bone marrow show an accelerated maturation of neutrophils and monocytes. Although no enhancement of endogenous respiration of bone marrow cells could be discovered, the influence of PMB considerably lowered the critical  $P_{O_2}$  level which limits respiration of the cell. That shows their ability to use oxygen more efficiently (61). Shifts similar in many respects were caused by triple injection of 30 mg of quartz suspension intraperitoneally and, to a lesser degree, by similar injection of  $\text{TiO}_2$ . This can be explained by formation of PMB *in vivo* in quantities depending on the cytotoxicity of dust.

Table 1. Effect of a single intratracheal injection of PMB on the number of free cells in the respiratory tract of rats.

Exposure	Intratracheal injection	Number of cells in washing $\times 10^6$			NL/AM
		All cells	AM	NL	
No exposure to dust	Normal saline	$4.47 \pm 0.51$	$3.02 \pm 0.53$	$0.39 \pm 0.14$	$0.13 \pm 0.03$
	PMB	$12.89 \pm 1.42^*$	$5.31 \pm 0.51^*$	$5.09 \pm 0.77^\dagger$	$0.96 \pm 0.01^\dagger$
Four ( $50 \text{ mg/m}^3$ ) exposures to $\text{TiO}_2$ , 5 hr	Normal saline	$8.66 \pm 1.20$	$6.46 \pm 0.71$	$1.56 \pm 0.77$	$0.21 \pm 0.10$
	PMB	$16.47 \pm 1.08^*$	$9.24 \pm 0.59^*$	$6.04 \pm 1.09^*$	$0.75 \pm 0.20^\dagger$

\*  $p < 0.01$ .

†  $p < 0.001$ .

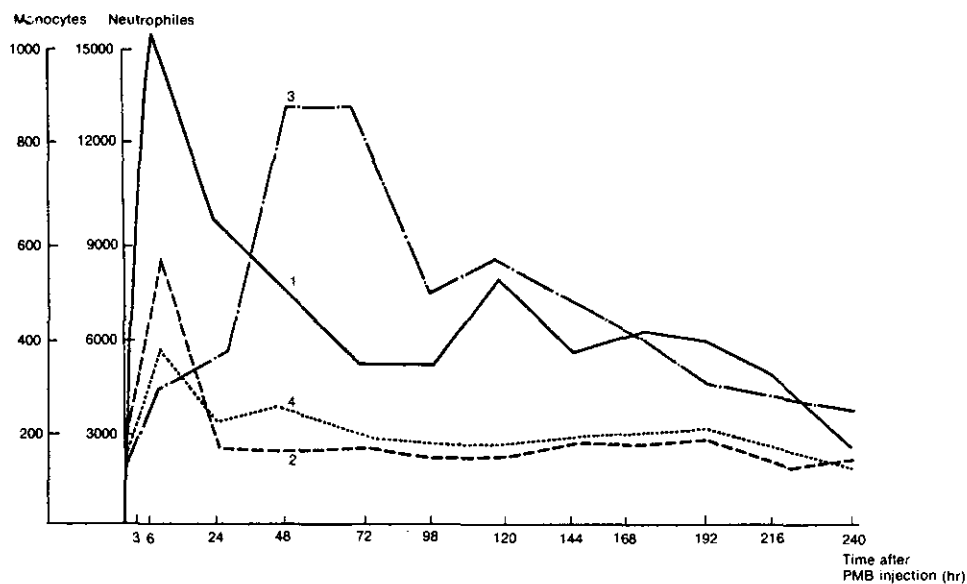
**Table 2.** Some cytological and polarographic characteristics of bone marrow cells of rats after three intraperitoneal injections of PMB, quartz, or TiO<sub>2</sub>.

	Normal saline	After intraperitoneal injection of		
		PMB	Quartz	TiO <sub>2</sub>
O <sub>2</sub> consumption, nA O <sub>2</sub> /min per 10 <sup>6</sup> cells	3.2 ± 0.3	3.14 ± 0.4	3.72 ± 0.8	2.95 ± 0.2
Critical level P <sub>O<sub>2</sub></sub> , mm Hg	38.3 ± 3.8	27.8 ± 3.2*	28.4 ± 2.1*	29.4 ± 4.5
Mature neutrophil count, % <sup>a</sup>	62.0 ± 4.3	77.0 ± 5.0*	82.5 ± 4.7*	67.0 ± 2.5
Monocyte count, % <sup>b</sup>	0.8 ± 0.1	2.1 ± 0.4*	1.3 ± 0.1*	1.3 ± 0.2*

\* Values statistically significantly different ( $p < 0.05$ ) from the corresponding ones after injection of normal saline

<sup>a</sup> Percent of mature forms in all the neutrophil series cells.

<sup>b</sup> Percent of monocytes in all the bone marrow cells (counting 1000 cells in each smear).



**FIGURE 1.** Effect of PMB on neutrophil and monocyte counts in peripheral rat blood: (1) neutrophils after injection of PMB; (2) neutrophils after injection of normal saline (control); (3) monocytes after injection of PMB; (4) monocytes after injection of normal saline (control). Differences between experimental and control series statistically significant ( $p < 0.05$ ) for neutrophils at all times of testing except the last; for monocytes from 24 to 144 hr.

Figure 1 shows the change of the average NL and monocyte counts in the peripheral blood of rats after a single injection of the same dose of PMB. The original sharp increases of the NL count is probably related to mobilization of neutrophils from different reserve pools, but the subsequent steady leukocytosis, both neutrophilic and monocytic, can be associated with the increase of granulocyte and monocyte formation.

Although PMB do not possess direct stimulating action on proliferation of stem cells *in vitro*, we observed an increase of CFU-S number with a shift of differentiation to granulocyte colonies after injecting PMB into both mouse bone marrow donors, and recipients.

### Influence of PMB on the Functional Activity of Cells Able to Phagocytize

Attraction of macrophages and neutrophils to the site of formation or injection of PMB can be imagined as a

result of motion of these cells against a concentration gradient of the factor which stimulates their migration. In fact, we found by means of a technique used for quantitative evaluation of macrophage migration (62) that PMB increases it. This effect depends on the PMB concentration in the medium.

The consumption of oxygen by alveolar and peritoneal macrophages (PM) was studied polarographically. On introduction of whole PMB to the cell-free medium, the oxygen was not consumed, and we have grounds for considering the observed increase in oxygen consumption under the stimulating action of PMB to be the result of a real enhancement of macrophage metabolism. When the number of cells in a polarographic unit was equal, the PMB stimulating effect was equal for AM and PM, in spite of the well-known peculiarities of aerobic metabolism of AM.

The decrease of the number of dust particles phagocytized by the single AM in lung washings from rats which received PMB intratracheally (Table 3) seems to be

Table 3. Effect of intratracheal injection of PMB on the activity of phagocytosis of dust particles *in vivo*.

Exposure	Intratracheal injection	"Active" AM, %	Average number of particles in "active" AM	"Active" AM with undefinable number of particles %	Average number of particles in NL
* None	Normal saline	70.3 $\pm$ 2.9	3.84 $\pm$ 0.99	Absent	1.67 $\pm$ 0.52
	PMB	49.1 $\pm$ 2.0†	2.85 $\pm$ 0.54*	Absent	1.50 $\pm$ 0.49 (NS) <sup>b</sup>
TiO <sub>2</sub> <sup>a</sup>	Normal saline	85.8 $\pm$ 1.9	Could not be counted	30.2 $\pm$ 5.7	1.43 $\pm$ 0.55
	PMB	77.0 $\pm$ 1.7*	Could not be counted	14.9 $\pm$ 4.2*	1.86 $\pm$ 0.55 (NS) <sup>b</sup>
TiO <sub>2</sub> <sup>a</sup>	Normal saline	72.4 $\pm$ 0.7	Could not be counted	11.1 $\pm$ 1.2	1.57 $\pm$ 0.39
	PMB	60.7 $\pm$ 1.8†	Could not be counted	0.6 $\pm$ 0.2†	1.82 $\pm$ 0.41 (NS) <sup>b</sup>

<sup>a</sup> Two different experiments.

<sup>b</sup> NS denotes differences between groups which are statistically not significant ( $p > 0.05$  according to Student's *t*-test).

\*  $p < 0.05$ .

†  $p < 0.001$ .

paradoxical in comparison with all the above evidence of functional stimulation of the macrophage under the direct influence of PMB. This effect can be seen by the average number of phagocytized particles in one active AM in rats which had inhaled dust only from the unfiltered ambient atmosphere, by the decrease of percent of active AM containing too many particles to count in rats which had in two independent experiments inhaled the TiO<sub>2</sub> dust in a chamber at different concentrations and by the lowering of the percent of active (i.e., containing at least one visible dust particle) cells of the general AM number in both groups.

One can suggest the following explanation for such a discrepancy between the stimulating action of PMB and the observed lowering of phagocytic activity of the free macrophage population of the respiratory tract. The intratracheal injection of PMB probably increases the recruitment of insufficiently mature cells from the interstitial pool of precursors, i.e., the contribution of these cells into the general population of macrophages entering the alveoli increases. This leads to the increase of the percentage of free AM which possess insufficient phagocytic activity in comparison with the mature AM functioning most efficiently in the peculiar conditions of free alveolar surface. The appearance of "young" and metabolically less active AM had been described also with quartz dust deposited in the lungs (41). Miller and Kagan (41) also paid attention to the fact that after quartz inhalation the free AM population is enriched by small, evidently immature cells. According to Grant et al. (63), smaller cells are recruited also when the total AM number in rabbits exposed to submicron Fe<sub>2</sub>O<sub>3</sub> aerosol inhalation was increased 3-fold. Kavet et al. (29), however, noticed an increase of macrophage volume and activation of phagocytosis *in vitro* during inhalation of the same aerosol by hamsters. Comparison of phagocytizing activity and immune properties of AM in smokers and nonsmokers also favors the idea that enhanced AM recruitment as a response to particle deposition leads to an increase in functionally immature cell percentage (64).

All the above agrees with the supposition that PMB

cause enhanced recruitment not only of mature AM, but also of those from the interstitial pulmonary pool which are morphologically and functionally immature. There are still more grounds to expect such a result if PMB also cause an increase of the direct influx of monocytes into the alveoli. On the other hand, for the NL there always exists only this route of recruitment into the alveoli, and, therefore, its enhancement under the influence of PMB must not decrease the average phagocytizing activity of NL washed out of the respiratory tract. As seen from Table 3, this does not occur.

The intratracheal injection of PMB simulates one of the results of deposition of cytotoxic dust particles in lungs. In real conditions of inhalation of such particles, when PMB are formed as a result of damage to phagocytizing AM by these particles, the above-mentioned effect means a decrease of the mean number of these particles in a single AM of subsequent generations whose recruitment would be connected with the breakdown of the first AM "echelons." This means a reduction of the cytotoxic action of dust on AM and, consequently, an increase of the probability of AM retaining its integrity and fulfilling its role in pulmonary clearance. The seemingly unfavorable PMB effect must receive other evaluation in the light of these ideas.

Further, due to a general increase in AM and NL counts, the total phagocytosis activity evaluated by the number of particles observed in a phagocytized state is not lowered but even considerably increased. This also contributes to the increase of clearance efficiency. In fact, after four daily dust exposures rats received 1 mL of normal saline intratracheally on the second and fourth day and were killed after 24 hr; the lungs of these rats showed on the average, 250.6  $\pm$  44.0  $\mu$ g Ti. Rats injected similarly with  $1.5 \times 10^8$  PMB showed only 151.0  $\pm$  21.4  $\mu$ g ( $p < 0.05$ ).

Thus, the favorable influence of PMB on the cellular pulmonary clearance mechanism, which serves as a partial compensation for the unfavorable influence related directly to the AM breakdown, consists in providing an effective engulfing of a greater total number of particles along with "defense" reduction of loading a single

macrophage by these particles. Both are favored by a partial redistribution of the total dust load in the sharply increased NL count.

## Role of the Lipid Fraction of PMB in the Control of Alveolar Phagocytosis

The supernatant obtained by centrifuging PMB under the conditions described above and the residue washed washed three times with normal saline showed practically the same NL and AM attraction when injected intratracheally into rats. Thus the recruiting activity of PMB is divided into two approximately equal parts by centrifuging, this half activity being subthreshold for AM recruitment in some, but causing both effects in other experiments (Table 4). Note that the whole product proved more active than the supernatant in the estimation of the action of PMB on migration *in vitro* as well. In both cases one may suppose that

phagocytizing of cell debris and additional lysis leads to freeing of the same acting agent which determines the activity of the supernatant.

The residue, as it was found, contains 1.5 times more protein than the supernatant, while the total lipids and different lipid fractions are divided equally between them. The supernatant obtained by analogous centrifuging of skeletal rat muscle which was first ground and then frozen and thawed three times did not influence the AM count when injected intratracheally, but gave a small increase of the NL count (Table 4). The injected dose of this product was equivalent in protein to the dose of the PMB supernatant which caused evident recruitment of both AM and NL. The total lipid content of this PMB dose was 17.7 times that in the muscle product. Let us note that the same product in a concentration which was equivalent in protein to a stimulating PMB concentration did not cause any change in oxygen consumption by peritoneal macrophages when introduced into the polarographic unit.

Even an evidently incomplete lipid extraction from

Table 4. Alveolar phagocytosis response 24 hr after the intratracheal injection of different products.<sup>a</sup>

Experiment number <sup>b</sup>	Intratracheal injection	Number of cells washed out of lungs $\times 10^6$			
		All	Alveolar macrophages (AM)	Neutrophil leukocytes (NL)	NL/AM ratio
1	Normal saline	0.66 $\pm$ 0.07	0.59 $\pm$ 0.06	0.014 $\pm$ 0.003	0.024 $\pm$ 0.006
	PMB	2.20 $\pm$ 0.10 <sup>+</sup>	0.88 $\pm$ 0.11 <sup>+</sup> <sup>x</sup>	1.21 $\pm$ 0.13 <sup>+</sup> <sup>x</sup>	1.36 $\pm$ 0.23 <sup>+</sup> <sup>x</sup>
	PMB residue	1.17 $\pm$ 0.11 <sup>x</sup>	0.45 $\pm$ 0.08	0.65 $\pm$ 0.08 <sup>+</sup> <sup>x</sup>	1.44 $\pm$ 0.31 <sup>+</sup>
	PMB supernatant	1.28 $\pm$ 0.05 <sup>+</sup>	0.68 $\pm$ 0.10	0.50 $\pm$ 0.08 <sup>+</sup> <sup>x</sup>	0.73 $\pm$ 0.16 <sup>+</sup>
	Lipids extracted by Folch mixture plus Tween 20	15.20 $\pm$ 2.98 <sup>+</sup> <sup>x</sup>	2.74 $\pm$ 0.96 <sup>+</sup> <sup>x</sup>	11.48 $\pm$ 2.38 <sup>+</sup> <sup>x</sup>	4.19 $\pm$ 1.71 <sup>+</sup> <sup>x</sup>
	0.2% Tween 20 in normal saline	0.72 $\pm$ 0.09	0.55 $\pm$ 0.07	0.10 $\pm$ 0.02 <sup>+</sup>	0.18 $\pm$ 0.04
2	Normal saline	0.95 $\pm$ 0.13	0.91 $\pm$ 0.13	0.013 $\pm$ 0.005	0.014 $\pm$ 0.006
	0.2% Tween 20 in normal saline	1.30 $\pm$ 0.09 <sup>+</sup>	1.19 $\pm$ 0.09	0.03 $\pm$ 0.013	0.025 $\pm$ 0.011
	Serum 1:10	0.99 $\pm$ 0.18	0.90 $\pm$ 0.16	0.018 $\pm$ 0.006	0.020 $\pm$ 0.008
	PMB supernatant	2.59 $\pm$ 0.49 <sup>+</sup>	1.92 $\pm$ 0.43 <sup>+</sup>	0.53 $\pm$ 0.24 <sup>+</sup>	0.28 $\pm$ 0.07 <sup>+</sup>
	Supernatant of the destroyed muscle	1.00 $\pm$ 0.07	0.83 $\pm$ 0.08	0.11 $\pm$ 0.05	0.13 $\pm$ 0.06
	Lipids extracted by Folch mixture plus Tween 20	43.28 $\pm$ 9.2 <sup>+</sup>	7.79 $\pm$ 2.80 <sup>+</sup>	33.76 $\pm$ 7.63 <sup>+</sup>	4.33 $\pm$ 1.84 <sup>+</sup>
3	Lipids extracted by Folch mixture plus serum	25.70 $\pm$ 10.40 <sup>+</sup>	8.95 $\pm$ 3.67 <sup>+</sup>	15.42 $\pm$ 7.20	1.72 $\pm$ 1.07 <sup>+</sup>
	Normal saline	0.94 $\pm$ 0.13	0.88 $\pm$ 0.12	0.011 $\pm$ 0.002	0.013 $\pm$ 0.002
	PMB	3.38 $\pm$ 0.23 <sup>+</sup> <sup>x</sup>	1.68 $\pm$ 0.13 <sup>+</sup> <sup>x</sup>	1.49 $\pm$ 0.12 <sup>+</sup> <sup>x</sup>	0.89 $\pm$ 0.10 <sup>+</sup> <sup>x</sup>
	Residue of PMB after ether treatment	1.57 $\pm$ 0.17 <sup>+</sup> <sup>x</sup>	1.19 $\pm$ 0.13 <sup>+</sup> <sup>y</sup>	0.28 $\pm$ 0.04 <sup>+</sup> <sup>x</sup>	0.24 $\pm$ 0.04 <sup>+</sup> <sup>x</sup>
	Lipids extracted by ether and Tween 20	37.03 $\pm$ 19.13 <sup>+</sup>	15.90 $\pm$ 8.25 <sup>+</sup> <sup>xy</sup>	18.82 $\pm$ 9.75 <sup>+</sup>	1.18 $\pm$ 0.87 <sup>+</sup>
	0.2% Tween 20 in normal saline	2.68 $\pm$ 0.45 <sup>+</sup>	1.53 $\pm$ 0.27 <sup>+</sup>	0.86 $\pm$ 0.16 <sup>+</sup>	0.56 $\pm$ 0.14 <sup>+</sup>

<sup>a</sup> Pairs of values compared at  $p < 0.05$  in the text are denoted by the same superscript symbols (x or y); the superscript symbol (+) denotes all values differing from control at  $p < 0.05$  (by Student's *t*-test).

<sup>b</sup> The PMB dose in experiments 1 and 2 corresponds to  $1.5 \times 10^8$  destroyed cells, and in experiment 3 to  $7.5 \times 10^7$  destroyed cells; the lipid dose always corresponds to the lesser of these PMB doses.

PMB (by ether, on cooling and shaking) gives a residue causing a less pronounced recruitment of NL and AM than the undefatted PMB (Table 4). The lipids extracted by the same method or fully extracted from PMB (Folch technique) and later reemulsified with Tween 20, proved much more active in this respect than corresponding doses of PMB. Even a simple addition of serum proteins to these lipids (instead of Tween 20) reduces this effect somewhat. We cannot exclude the possibility that the paradoxically high activity of extracted macrophagal lipids is related to a fuller freeing of lipids from lipoprotein complexes than that during simple macrophage breakdown. However, when we studied the macrophage migration *in vitro*, the same lipids proved to be only just as active stimulants of migration as corresponding quantities of PMB supernatant. One may suppose that in a closed system of cell culture the action of proteolytic enzymes, leading to complete liberation of lipids from lipoprotein complexes, evens out effects of PMB and of extracted lipids as recorded after 24 hr.

The stimulating action of triglycerides on macrophages *in vitro* was also described independently (65). Russell (66) discovered a lipid chemotactant for monocytes and macrophages in a medium conditioned by *Corynebacteria*.

There are also other proofs of the fact that chemotaxis of alveolar macrophages may be caused by lipids (67), in particular by material rich in lipids lining the alveoli (68). The latter is physiologically justified by the fact that one of the important functions of the AM in the normal state (i.e., even in the absence of any particles deposited in lungs) is the disposal of pulmonary surfactant. It is possible that it is the constant secreting of the latter by Type II cells of alveolar epithelium which is the main cause of the "background" level of free AM always observed in the lungs.

In the whole, all these data agree with the supposition that some lipid substances freed during macrophage breakdown under the action of silica play an important role in stimulation of alveolar phagocytosis (39). Civil and Heppleston (69) showed that intravenous injection into rats of lipids extracted from rat lung tissue of rats in which quartz dust inhalation caused the development of alveolar lipoproteinosis, stimulates DNA synthesis by cells which are precursors of monocytes in bone marrow. The authors see in this a confirmation of the key role of hemopoiesis in replenishment of the population of pulmonary macrophages broken down by deposited dust. Lipids produced as a result of contact with quartz by alveolar epithelium Type II cells, or, possibly, by alveolar macrophages, are the stimulators of this process.

Some reasons why we hold another opinion on the origin of lipids in pulmonary macrophages are given below. One may consider most probable that a systemic hemopoiesis response to local macrophage breakdown is only the last reserve of their supply used when aggression is most massive and prolonged. When the demands are less, they are satisfied by the local pool of proliferat-

ing interstitial macrophages. With these stipulations the data of Civil and Heppleston fully agree with our views on the role of macrophagal lipids in autoregulation of alveolar phagocytosis.

## Macrophagal Lipids and Phagocytosis of Quartz Particles

Quartz dust inhalation causes a considerable increase of sudanophilic inclusions in pulmonary macrophages, especially when fat is introduced into the stomach of rats in increased quantities (19,70,71). This shift may be associated with a stimulation of the lysopectic function of the interstitial pool of pulmonary macrophages.

Another mechanism of formation of these inclusions may be the engulfment by alveolar macrophages of the phospholipid pulmonary surfactant which is actively produced by the Type II epithelial cells when quartz particles are deposited. The enhancement of secretory function of the pneumocytes is, supposedly, considered as a result of the action of some products liberated by the same macrophages under the cytotoxic action of quartz (72).

We showed that intraperitoneal injection of quartz does not influence the number of sudanophilic inclusions and content of different lipids in peritoneal macrophages, i.e., in cells not taking part in the lypopexy process. As far as the stimulating action of quartz on fat engulfment by pulmonary macrophages is concerned, it can also be explained by the action of PMB. We showed in a special experiment that the percent of AM containing a high number of sudanophile inclusions sharply rises 24 hr after intratracheal injection of the PMB supernatant.

It is natural to suppose that the breakdown of such AM under the influence of quartz particles must lead to formation of PMB with an increased lipid content which, as shown above, is associated with the stimulating action of PMB in recruitment of phagocytizing cells. Thus, a positive feedback between the cytotoxic action of quartz and the phagocytotic response of the lungs is realized not only through an increased amount of PMB, but also further through an increase in PMB activity (Fig. 2). Moreover, it was shown that viable human alveolar macrophages, when activated by appropriate stimuli (73,74), release a neutrophil chemotactic factor which is of low molecular weight and at least partially lipid in nature. If one keeps in mind that PMB do activate viable macrophages, one may surmise an additional positive feedback from destruction of AM by silica cytotoxicity to recruitment of NL into airways, operating through the above factor. This feedback is not shown in our scheme which had been formulated before those data were published. One more positive feedback, shown in the same scheme, reflects the earlier proved (19,71) enhancement of alveolar macrophage degeneration caused by inhaled quartz in rats, which had, due to long-term excessive fat administration PO, an increased sudanophilic inclusion content in AM.

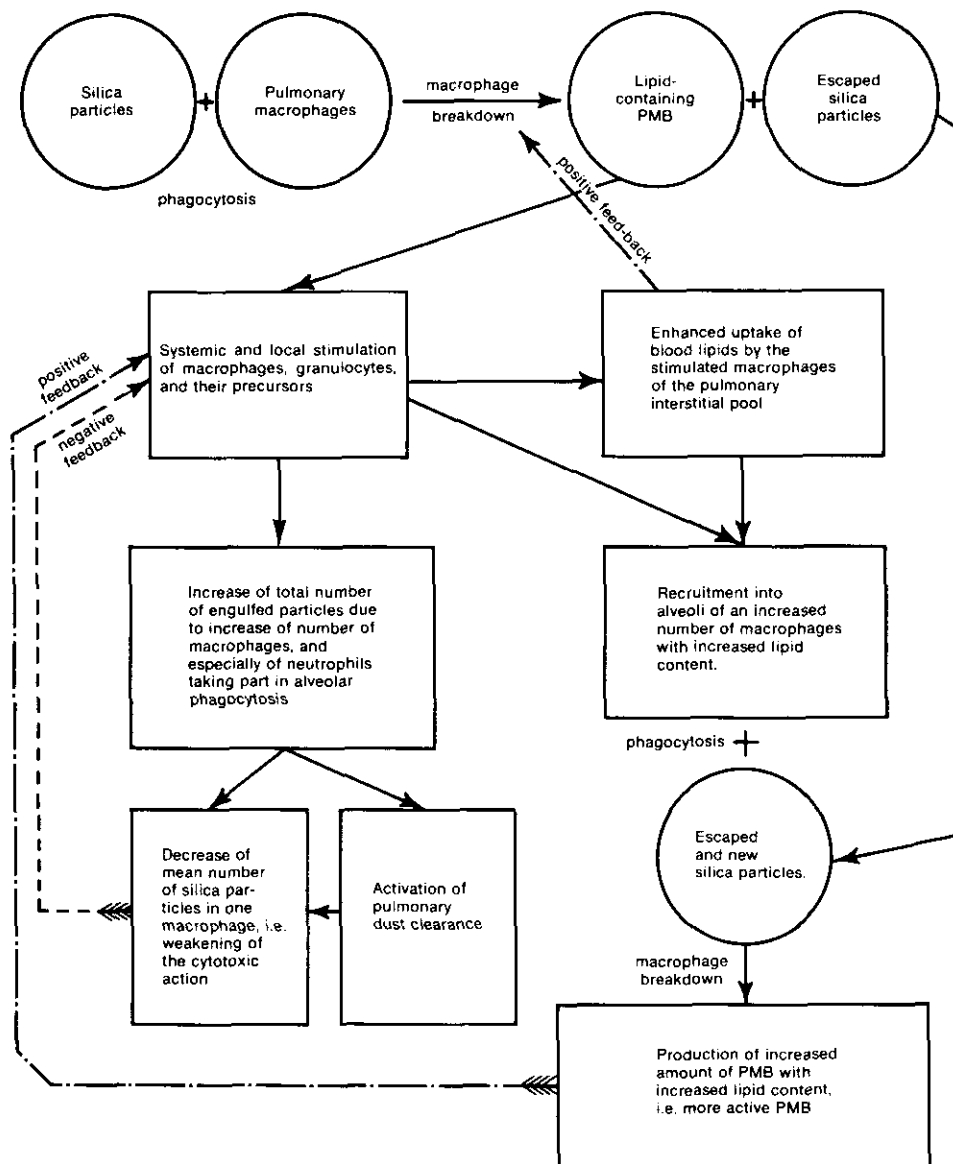


Figure 2. Control of the alveolar phagocytosis response to deposited silica particles by the macrophage breakdown products (PMB).

Together with this, there soon appears a negative feedback, the essence of which is not only in clearance of the cytotoxic particles from the lung, stimulated by the same PMB, but even earlier, in a redistribution of the particles between mature and immature AM and NL which promotes the weakening of the cytotoxic effect. As a result, the number of newly formed PMB begins to decrease gradually, which leads to a gradual lowering of the number of cells again recruited to replace those cleared from the respiratory tract.

## Autocontrol and Control

Two long-term experiments showed that the AM and NL recruitment could be enhanced by cold acclimatiza-

tion in spite of the fact that it increased the resistance of AM to the cytotoxic action of quartz, i.e., the mean percentage of degenerated AM was lowered, which fully corresponded with the decrease in NL/AM ratio (17). In other cases of induced increase of AM resistance, when the rats were physically trained, exposed to long-term inhalation of sulfur dioxide or injected with poly (vinylpyridine *N*-oxide) or a pharmacological adaptogen of the benzimidazole derivative series in our laboratory, we observed not only a lowering of this ratio, but also a general decrease of the number of recruited cells (17, 18) which can be easily explained by a decrease in the PMB formed. Acclimatization to cold in rats not inhaling quartz did not by itself cause any recruitment of cells into the respiratory tract. The dependence of

pulmonary dust clearance and/or of alveolar macrophage recruitment on cold or other stresses is also evidenced by some other experimental data (75-77). Consequently, there can exist some other mechanisms of control, besides the autoregulation of the alveolar phagocytosis through PMB and sometimes even seemingly against it.

A possible increase in macrophage recruitment may occur under the influence of atropine, epinephrine, or estrogens (78). This recruitment may be depressed under the influence of serotonin (33), a distant inflammation site (79) and glucocorticoids, which also depress the phagocytizing ability of macrophages (29,80). However, many data referring to this question are contradictory. Domby and Whitcomb (81) found no changes either in number or in immune functions of alveolar macrophages in guinea pigs under the action of cortisone, while according to data obtained by Lupu and Velican (78) and a number of other researchers the same hormone, even if it depresses lung macrophage recruitment, stimulates their phagocytic activity.

On the whole, the data accumulated hardly permit discussion of any consistent system of ideas about the neurohumoral control of phagocytic responses in general and the alveolar phagocytic response in particular. We believe that the autoregulation of this response can be considered to be the main one, while two points of conjugation of this autocontrol with the action of specialized control systems of the organism are possible. First, some hormonal influences can probably change the AM resistance to damaging influences or else change the activity of engulfing of cytotoxic particles by AM; in both cases they influence the amount of PMB formed. Second, hormonal influences can change the reaction of different elements of the phagocyte response to the action of the same amount of PMB. In particular, we found that both the total number of cells recruited into the respiratory tract and the NL/AM ratio, as well as the influence of PMB on the number of dust particles phagocytized by these cells, change considerably if the PMB is injected intratracheally 18 hr after an intramuscular injection of hydrocortisone.

In short, this change consists in the fact that the NL/AM ratio is decreased (compared to the action of PMB on control rats) by some decrease of the number of NL while the number of AM increases considerably. The percentage of active phagocytes and the average number of phagocytized dust particles proved to be considerably higher for both cell types. We must note that the same dose of hydrocortisone in the experiment performed by Kavet et al. (29) on hamsters inhaling iron oxide, suppressed recruitment of both macrophages and neutrophils while phagocytic activity of alveolar macrophages *in vitro* was weakened.

Iron oxide is notable by its very low cytotoxicity. Contradictions between our results and those of Kavet et al. may therefore serve as indirect evidence of the fact that the total effect depends not only on the hormonal background, but on the strength of the

autoregulating stimulus acting on this background as well. This problem certainly requires further study.

In another experiment we reproduced an inhibition of the NL recruitment by hydrocortisone although there was no increase of the AM recruitment. In another group of male rats injected IM with estrone as a mineralocorticoid hormone the response of NL to a given dose of the PMB was, on the contrary, enhanced.

A group of atropinized rats challenged with the PMB responded with a dramatically decreased recruitment of NL, and proserine injected after atropine (but before the PMB) did not change this picture. On the contrary, rats injected with proserine after a sham premedication (with normal saline SC) showed a significant increase of NL recruitment. To test the role of the adrenergic control, we conducted a similar experiment using ephedrine as an adrenomimetic drug and dihydroergotoxine as a drug with predominantly  $\alpha$ -adreno-blocking action. It was shown that the former suppressed the recruitment of NL by the PMB while the latter either enhanced it (if injected before SC injection of the normal saline) or prevented the suppressing effect of the ephedrine injected after dihydroergotoxine.

Thus, both corticoid hormones and autonomous nervous stimuli of antagonistic general action can shift in opposite directions also the host's sensitivity to regulating action of products of the macrophage breakdown on alveolar phagocytosis. A conclusion seems to be justified that, at least, the recruitment of NL into airways, i.e., the most important feature of the pulmonary phagocytic response to deposition of cytotoxic particles, depends not only on the degree of their cytotoxicity but also on the reactivity of organism to the action of the PMB, this reactivity being subjected to a neurohormonal regulation.

## Conclusion

A transient increase of the number of phagocytizing cells recruited into the lower respiratory tract is an obligatory response to a sufficiently considerable deposit of inhaled or injected particles. A number of indirect arguments proves the important role of this recruitment as a physiological mechanism of pulmonary dust clearance. This response depends quantitatively both on the number of particles deposited and on their cytotoxicity. This last property of dust also determines important qualitative characteristics of the process considered and the contribution of neutrophilic leukocytes into the alveolar phagocytosis of particles.

Such an adaptation of this defense mechanism to quantity and properties of inhaled particles, as well as its close dependence on intergroup and interindividual variations of macrophage sensitivity to the cytotoxic action of the dust are in favor of the autoregulation of the process depending on intensity of cell breakdown caused by this action. It was experimentally proved that macrophage breakdown products (PMB) stimulate the functional state of macrophages and neutrophils *in*

*vitro*; increase their recruitment into the lungs, simultaneously causing a dose-dependent increase of neutrophil count to alveolar macrophage count ratio (which, most probably, promotes the elimination of cytotoxic particles) and promote the recruitment of corresponding cell reserves of the organism and signal the necessity of their replenishment by increased monocyte- and granulocyte poiesis. It was proved that these effects are caused, at least in part, by the influence of macrophagal lipids whose accumulation in macrophages is, in its turn, increased under the influence of PMB.

The suggested mechanism of autoregulation may be considered the basis of an adequate control of alveolar phagocytosis as a defense mechanism. Data obtained by intraperitoneal injection of PMB allow us to think that a similar mechanism plays a definite role in phagocytosis regulation in general. However, the dependence of this reaction on neurohormonal influences is also evident, and there are some reasons to suppose that they define the intensity and, sometimes, even the direction of the response to the action of PMB, as well as the intensity of PMB formation.

Induced stimulation of phagocyte recruitment into the respiratory tract can promote, to a certain degree, pulmonary dust clearance. However, hyperstimulation or stimulation on the background of a response whose high intensity is already assured by autocontrol can lead to an opposite effect. On the whole, this method is risky enough as a prophylaxis measure, especially when the influence of dusty air lasts many years.

A number of important questions connected with the problem cannot as yet be convincingly answered and deserve further active studies. In the first place, among them are finding direct proofs of this or that particular mechanism of participation of alveolar phagocytosis in lung clearance, as well as the question of interdependence between the auto and systemic regulation of alveolar phagocytosis.

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